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Emerging Use of Nanoparticles in Diagnosis Of Atherosclerosis Disease: A Review

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Abstract. Despite over the last decades, cardiovascular research has significantly enhanced our understanding of the atherosclerotic process, the molecular mechanisms underlying the pathology remain mostly unclear. In fact, the current diagnostic modalities do not inform clearly on the cellular and molecular processes that drive the development of atherosclerotic pathology and each of the imaging techniques has advantages and limitations in terms of radiation exposure, reproducibility, sensitivity, resolution and costs. Therefore, to overcome the limitations of individual imaging modalities and develop novel and preventive strategies, we need additional approaches to detect the atherosclerotic plaques' formation in patients at high risk for clinical events. In this context, a new dimension of information lie in the molecular imaging that allows to get a better knowledge of biological phenomena. In addition, multimodal imaging approaches play a pivotal role for the earlier detection of pathological process. Here, we provide a critical analysis of the various imaging modalities currently used in clinical and nanotechnology approaches based on the development of bio-nanocarriers for detection of cardiovascular diseases.

ATHEROSCLEROSIS AND DIAGNOSIS

Cardiovascular disease continues to be the leading cause of death in the Western world [1] and it is caused mainly by atherosclerosis. It is a multifactorial systemic disease characterized by arterial wall thickening and rigidity and the formation of the characteristic plaques that developed simultaneously in medium and large-sized arteries, inducing a blood flow reduction with different complications [2]. This inflammatory pathology that has origins in childhood and occurs decades before the disease becomes clinically apparent (cardiac arrest, acute myocardial infarction or stroke) [3,4]. The pathogenesis of atherosclerosis has been the subject of many scientific works and the major players involved in this process are endothelial cells, inflammatory and immune cells (mainly macrophages and T cells), and intimal smooth muscle cells (SMCs) [5,6]. For many years it was believed that the disease was only characterized by a passive accumulation of cholesterol in the vessel wall, but, nowadays, it is known that the evolution of the lesion is much more complex and not fully clarified. In addition, the degree of luminal stenosis is only indirect indicator of atherosclerotic process [7].

At the beginning, our understanding of the atherosclerotic pathology is mainly based on postmortem examinations of human coronary arteries or analysis of resected surgical specimens from patients who underwent carotid endarterectomy. In recent years, several imaging techniques, invasive and noninvasive, are available to detect and display different characteristics of atherosclerotic lesions of clinical interest [8]. The choice and applicability of each imaging technique depend not only on its diagnostic efficacy but also on the type of questions being asked. Unfortunately, these imaging modalities, neither characterize nor correlate the image parameters with histopathological lesion types, which are more clinically relevant. Most of the standard imaging modalities characterize some of the morphological and functional features of the vascular lesion, but a quantitative evaluation

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of atherosclerotic disease during its natural history and following therapeutic interventions are necessary for understanding the stabilization or progression of the disease and for selecting suitable medical or surgical interventions.

This work highlights the latest knowledge about the role of imaging and future research directions based on nanotechnology approaches in atherosclerosis diagnosis.

IMAGING TECHNIQUES FOR ATHEROSCLEROSIS

Angiography

Initially, contrast angiography has been the gold standard imaging technique for atherosclerosis, providing information about site and severity of luminal stenosis, but it is unable to detect atherosclerotic lesions that do not protrude into the lumen and does not give information about plaque composition or vulnerability [9].

Unfortunately, the positive remodeling does not allow to assess the integrity of the arterial lumen in case of cardiovascular disorders subclinical. Another limitation of angiography is that underestimate the degree of local stenosis. Furthermore, it is an invasive technique which can cause complications, is highly observer dependent and not very reproducible [9,10]. For all these reasons, other imaging modalities have been examined for the atherosclerotic process.

Intravascular Ultrasound (IVUS)

Currently, the use of Intravascular Ultrasound (IVUS) is confined to research or for interventions (ie. angioplasty), because it is invasive technique [4]. This imaging modality allows an in vivo characterization with a resolution [11] of around 10 μm and direct assessment of coronary plaque volume and plaque morphology delineating the thickness and vessel wall structures [12].

Thus, IVUS allows tomographic assessment of lumen area and the composition of atherosclerotic plaque, whereas Angiography depicts a two-dimensional (2D) of the lumen vessel. In summary, IVUS is able to visualize atheromatous plaques which are angiographically invisible [13]. An important application of IVUS is the potential identification of high-risk plaques of rupture.

Magnetic Resonance Imaging (MRI)

Magnetic Resonance Imaging (MRI) is a highly flexible, reproducible and noninvasive imaging modality. In contrast to the other medical imaging methods which expose patients to ionizing radiation, MRI uses strong non-ionizing electromagnetic fields in the radio frequency range, offers excellent spatial resolution, is not operator dependent, and provides 3D data [14]. Compared to other imaging techniques (i.e. nuclear medicine techniques), it shows low sensitivity and long acquisition time [15]. The use of contrast agents (CAs) are often required in MRI scans in order to improve the enhancement of MRI signals [16].

This technique allows evaluating the arterial vascular tree and its use in the diagnosis of carotid atherosclerotic process has been validated and allows to obtain detailed information about the macrostructure of the plaque [14]. In addition, MRI is capable of discriminating between different component of plaque, such as lipid core, fibrous cap, intraplaque hemorrhage and calcification, and has the potential to identify vulnerable plaque before an ischemia [3]. Generally, these elements appear as isointense (ie. Lipid component on T1-weighted sequences), others hypointense (ie. calcium within the plaque on T1- and T2-weighted sequences) or hyperintense (ie. the fibrous cap) [17].

The carotid arteries are vessels of good caliber that are not subjected to movement. Thus, they are ideal for noninvasive imaging studies. In contrast, the motion artifacts, the reduced size, the position and the tortuosity of the coronary arteries make their visualization technically difficult [14]. Evaluation of the arterial wall , although of great clinical utility, turns out to be technically more difficult. Furthermore, the thickness of the coronary artery wall correlated with the temporal progression of the medical signals remains to be investigated.

Computed Tomography (CT)

Computed Tomography (CT) is a noninvasive imaging modality and is useful for evaluation of the arterial calcification, an early indicator of atherosclerosis. It provides a high temporal and spatial resolution and allows detailed anatomical visualization of atherosclerotic coronary disease and other cardiac abnormalities in medium and large-sized vessels [8]. Currently, the technical limits of CT are: poor soft tissue contrast and poor identification of subcomponents plaque. In addition, unlike MRI, CT exposes patients to high radiation dose and the ionated CAs [18].

CT scans can be performed with or without administration of contrast agents. In the first case, the diagnostic technique allows detecting non-calcified plaque components, while, in the second case, the coronary calcium [19].

Positron Emission Tomography (PET)

Unlike CT or MRI, which show anatomic detail, Positron Emission Tomography (PET) is a noninvasive nuclear imaging technique and provides quantitative in vivo assessment of physiological and biological phenomena. This modality necessitates the injection of a small quantity of radioisotopes used as tracers [20]. PET agents provide a better functional assessment of atherosclerotic plaques than tracers used in current nuclear imaging modalities.One of the most used PET agents, for understanding the pathological stages of vascular lesion in vivo, is 2-deoxy-2-^{18}F-fluoro-D-glucose (^{18}F-FDG), a synthetic molecule that competes with glucose for uptake into metabolically active cells (ie. inflammatory cells), but is not metabolized [21]. The advantage of PET over other imaging modalities, such as SPECT (Single Photon Emission Computed Tomography), is represented by high spatial resolution and contrast resolution and superior sensitivity that allow detection of picomolar tracer concentrations in the arteries [22]. Unfortunately, limited spatial resolution (~ 2 mm) means that images must be coregistered with CT or MRI for precise anatomical localization of ^{18}F-FDG uptake. Several clinical and preclinical studies of atherosclerosis show that a high macrophage density correlates with enhanced ^{18}F-FDG uptake in vessels with plaque. Other works, instead, have demonstrated that the increased uptake of ^{18}F-FDG, a transient phenomenon, in the large arteries of patients correlated with atherogenic risk factors. Furthermore, this technique is not yet standardized and ^{18}F-FDG uptake or changes in ^{18}F-FDG uptake, correlated to cardiovascular events, can generate false positives or negatives [23].

NONINVASIVE MRI MOLECULAR IMAGING

As described previously, MRI represents the unique technique that combines excellent soft tissue discrimination with high spatial resolution without the use of ionizing radiation. Nevertheless, this imaging modality is limited by its low sensitivity and requires the use of CAs to display the atherosclerotic plaques clearly. This restriction may be overcome with a noninvasive molecular imaging approach, considered an in vivo equivalent to immunohistochemical techniques and based on a signal imaging element encapsulated or conjugated to a carrier that transports a ligand that is then recognized by the target molecule. In fact, this strategy can facilitate early diagnosis, has the potential to image the pathophysiological process of the disease before the onset of symptoms and can be applied to follow the efficacy of therapy. In this scenario, the advantage of MRI resides in its ability to provide not only anatomical but also functional information quantifying specific biological processes within a single imaging modality. A variety of molecular targets has so far been successfully employed in preclinical models of cardiovascular disease to identify typical features associated with plaques that are prone to rupture. Examples of biomarkers are shown below: cell adhesion molecules (VCAM 1 or E-selectin) [24], extracellular matrix, lipoproteins, smooth muscle cells, macrophages, phosphatidylserine and αVβ3 integrin [25,26]. At first, the CAs were conjugated with monoclonal antibodies or specific peptides, but excellent results are then obtained with nanoparticles (NPs) that combine a high binding affinity for the target zone with the capacity to transport a sufficient amount of a contrast agent. The most widely employed NPs are: superparamagnetic iron oxide (SPIO), micelles, liposomes dendrimers and polymeric nanoparticles [26-28]. Table 1 summarizes the main diagnosis applications of these nanovectors. In this section, we will focus on recent progress in the use of nanoparticles for molecular MR imaging in atherosclerosis disease.

TABLE 1. *Nanovectors for diagnostic applications*

Nanocarrier	Pros	Cons	Example	Target	Application	Ref
Micelle	Biocompatibility Biodegradability Easy chemical modification	High instability Cannot be stored Must be made fresh	(MDA2, E06, and IK17)-labelled Gd-micelles	OSE (Active targeting)	Oxidative Stress	[54]
Dendrimer	Size and shape controllability Water solubility Biocompatibility	Non-specific Cytotoxicity Rapid clearance	MDA2-labeled MnDTPA-G8 PAMAM dendrimers	OSE (Active targeting)	Oxidative Stress	[70]
SPION	Robustness and stability Resistance to enzymatic degradation Long circulation time	Non-biodegradability Limited biocompatibility	(VHPKQHR)-labelled MIONs	VCAM-1 or E-selectin (Active targeting)	Endothelial activation	[24, 40]
			USPIOs	Macrophages (Passive targeting)	Inflammation	[34-37]
			SPIONs coated with dextran	PS (Active targeting)	Apoptosis	[39]
Liposome	Easy conjugation and functionalization Rapid cellular uptake Biocompatibility	Rapid clearance High production costs Low solubility	Gd-containing PS liposomes	VCAM-1 or E-selectin (Active targeting) Macrophages (Passive targeting) PS (Active targeting)	Endothelial activation Inflammation Apoptosis	[62]
Polymer	Water solubility Non-toxicity Biodegradability	Time-consuming preparation processes Expensive equipment	Gd-loaded PLGA/RGD	RGD Sequence (Active targeting)	Thrombosis	[51]

ABBREVIATIONS: ***Gd****, Gadolinium;* ***USPIOs****, ultrasmall superparamagnetic iron oxide nanoparticles;* ***SPIONs****, superparamagnetic iron oxide nanoparticles;* ***VCAM-1****, vascular cell adhesion protein 1;* ***DTPA****, diethylenetraminepentaacetic acid;* ***PS****, Phosphatidylserine;* ***OSE****, Oxidation-specific epitopes;* ***Mn****, manganese;* ***MIONs****, monocrystalline iron oxide nanoparticles;* ***PAMAM****, poly(amidoamine);* ***RGD****, arginylglycylaspartic acid;* ***MDA2****,* ***E06*** *and* ***IK17****, oxidation-specific antibodies;* ***VHPKQHR****, linear peptide for VCAM-1;* ***PLGA****, poly(lactic-co-glycolic) acid.*

Superparamagnetic Iron Oxide Nanoparticles (SPIONS)

Several MRI strategies to display the atherosclerotic lesions were successfully developed using NPs platform [29,30]. In effect, their chemical, physical and pharmacokinetic characteristics and the ability to transport high payloads make them highly suited to cellular and molecular imaging of atherosclerotic lesions.

Generally, two categories of contrast agents are used for molecular MR imaging of atherosclerotic plaques: superparamagnetic iron oxide nanoparticles (SPIONs) and nanoparticles that incorporate gadolinium (Gd) chelates [27,31]. SPIONs represent the main platform used and are composed of an iron oxide core formed by magnetite (Fe_3O_4) and/or maghemite (γFe_2O_3) and coated with a polysaccharide, synthetic polymer, or monomer, which make them water soluble, prevent their aggregation and improve biocompatibility [32]. Moreover, the combination "core-shell" influences the pharmacodynamic and pharmacokinetic features of the final product. Passive targeting of these nanocarriers dependent on the control of parameters such as the surface charge and hydrodynamic radius that affect circulation time of the nanoparticles, accessibility to tissues, opsonization, and so on. Differently, active targeting takes advantage of nanoparticle' surface modifications with monoclonal antibodies or peptides [32]. Generally, the term SPIO can be used to refer to: standard SPIO (SSPIO, 60-150 nm), ultrasmall SPIO (USPIO, <50 nm), monocrystalline iron oxide nanoparticles (MION,~30 nm) and cross-linked iron oxide (CLIO) [33]. Phagocytic cells of reticuloendothelial system (RES) take up injected SPIONs spontaneously by endocytosis or phagocytosis allowing a rapid accumulation of these particles at the level of the lesion of interest [34]. This system have been characterized as MRI contrast agents for the imaging of the plaque inflammation, which represents one the most of the features of high-risk atherosclerotic plaques [29]. In particular, Ruehm and coworkers [34] demonstrated that in hyperlipidemic rabbits there is an accumulation of USPIOs in plaques with high macrophage content and that this phenomenon induced MR signal changes. For this reason, Kooi and colleagues [35] investigated the detection of macrophages in human atherosclerotic plaque. The results showed that the use of a USPIO agent, Sinerem® (Guerbet; Ferumoxtran-10), accumulated mainly in macrophages in human atherosclerotic lesions prone to rupture, it induced significant decrease of signal T2* images obtained 24 hours after intravenous administration but not in the images obtained after 72 hours (washout phenomenon). This information suggested that USPIO-enhanced MRI is as a promising method for the in vivo differentiation between low- and high-risk plaques and additional studies conducted by Trivedi et al. [36] confirmed these preliminary results, suggesting furthermore that there is a process of accumulation and excretion of USPIOs.

A representative example of a study, in which MRI is used to monitor the target site accumulation of USPIOs, is published by Tang and colleagues [37]. In summary, the researchers explored whether there is a difference in the degree of inflammation between asymptomatic and symptomatic patients.The results suggested that one inflamed symptomatic vascular bed can be increase the risk of other arterial vessels to become inflamed. Finally, preclinical (atheromatous rabbits and ApoE knockout mice) and clinical studies of Sinerem® for noninvasive MRI assessment of atherosclerotic plaque inflammation are summarized by Tang et al. [38].

An example of active targeting, for development of a non-invasive method to detect vulnerable plaque prior rupture in vivo, is reported by Smith et al. [39]. In this investigation SPIONs consisting of an iron oxide core coated with dextran and conjugated to a cellular protein, Annexin V, that recognizes apoptotic cells by specific molecular interaction with Phosphatidyl Serine (PS). They tested in two rabbit models of atherosclerosis and MRI was performed with a 4.7 T small animal MRI system. The results were confirmed by further histological investigation and vascular targeting by the system, SPIONs-Annexin V, was atheroma-specific. In addition, the administered dose was significantly lower than the particles without target in the same animal model. Therefore, the presence of a biomarker, as Annexin V, can provide additional support for the diagnosis of vulnerable plaque.

Nahrendorf et al. [24], instead, functionalized MION with linear peptide (VHPKQHR) for targeting of the vascular cell adhesion molecule-1 (VCAM-1), which is a biomarker expressed at early stages and progression of atherosclerotic lesions. Even in this case, the conducted studies in animal models showed that the anatomical area of interest became dark (hypointense signal) after the injection of the nanoparticles. Kang and colleagues [40] prepared similar system using CLIO nanoparticles with E-selectin antibody fragments to detect E-selectin in endothelial cells. The expression of this molecule is induced by an inflammatory cytokine (interleukin-1β) and, as expected, a high decrease in T2* signal is present in the treated mice with interleukin-1β compared to mice not treated.

Many research groups have long studied the use of these carriers based on the models mentioned above in atherosclerosis detection and several scientific works are reported in the literature [41], but none is currently approved for clinical diagnostic evaluation and there are not others in clinical development.

Polymeric Nanoparticles (PNS)

Recent progress in synthetic polymer chemistry have produced a plethora of polymeric nanoparticles (PNs) in nanomedicine field for diagnostic applications [42, 43]. PNs can be made from organic polymers or inorganic materials and according to their intended application, nanoparticles can be engineered to impart the required properties [44]. Biopolymer nanoparticles can give several advantages: biodegradability, biocompatibility, effective encapsulation of active molecules, long circulation half-life, controllable size (sub-micron) that permits biodistribution different to the small molecules and easy surface functionalization for delivery to the site of interest [45]. Therefore, the versatility of these structures makes them an attractive platform for developing molecular imaging agents. The strategy to prepare PNs with imaging functionality is to incorporate materials or functional groups with some characteristic that makes them a new promising tool for the diagnostic. Generally, the CA can be covalently conjugated or physically encapsulated within polymeric matrix [46-49]. In the first case, the molecules with imaging properties are connected to polymeric backbone and there may be nonhomogeneous distribution and poor loading efficiency of CA on the polymer surface. Conversely, in the latter case, the system offers high loading efficiency and homogenous distribution of contrast media within the polymeric matrix. Initial characterization of polymeric nanoparticles containing gadolinium chelate (Gd-DTPA) as CA for enhanced MRI is reported by Doiron et al. [50]. In this work a water-in-oil-in-oil double emulsion solvent evaporation technique was used to encapsulate the CA in a poly(lactide-co-glycolide) (PLGA) or polylactide-poly(ethylene glycol) (PLA-PEG) particle for the transport of MRI agent for the detection of staged atherosclerosis. PLGA particles showed negative zeta potentials, while PLA-PEG particles had neutral zeta potentials. In vitro experiment showed that cytotoxicity of these particles on human umbilical vein endothelial cells (HUVEC) was minimal, while MRI in vitro experiment demonstrated that the relaxivity of the PLGA particles is similar to that of unencapsulated Gd-DTPA. Recently, Zhang and colleagues [51] have successfully synthesized using water in oil in water method and characterized a new type of delivery system based on PLGA. In this case, (Gd)-loaded PLGA nanoparticles show on the surface a specific peptide sequence (Arg-GlyAsp-Ser, RGDS) for the detection of thrombus at the molecular level. The results of in vitro experiments suggest that these molecular probes can be used for detection of thrombus with a longitudinal relaxation similar to commercial CAs.

Recently, our group is focused on the use of biomaterials to improve the healthcare services in the field of MRI and potentially for atherosclerosis diagnosis. Russo et al. [47], for example, report a new Hyaluronic Acid (HA) nanoprobe (35 nm), obtained by a controlled and continuous microfluidic process, which entraps CAs for MRI. In a subsequent work, the impact that hydrophilic biopolymer networks have on the relaxivity of Gd-based CAs has been analysed and the concept of “Hydrodenticity” has been defined to describe the ability of these biopolymers to enhance the properties of the metal chelate, as reported by Ponsiglione et al [48]. Vecchione et al. [49], instead, describe a core-shell architecture for multimodal imaging applications obtained by a modified complex coacervation. The relaxivity of Gd-DTPA nanoconstructs is more than four times higher than the relaxivity measured for free Gd-DTPA in solution.

Micelles

Micelles are self-assembled nanostructures composed by amphiphilic molecules (lipid or polymer). They can be made mainly by a hydrophobic core and externally a hydrophilic surface, characteristics that allow encapsulating therapeutic or diagnostic agents within the micelles. A first in vitro study is conducted by Lipinski et al. [52] that evaluated the uptake of micelles linked to specific antibody (immunomicelles) for macrophages and containing Gd-DTPA micelles, and a murine model of Apolipoprotein E knockout (ApoE KO) is used for ex vivo imaging of lesions. The micelles (size <100 nm) are made by lipid monolayers and the results of the experiments demonstrated that the immunomicelles are taken up by the macrophages compared to untargeted micelle and both micelles and immunomicelles are superior CAs compared to the others used in clinical practice. This enhancement is related to the content of macrophages, which is associated with plaques vulnerable to rupture. A limitation for this study is represented by long acquisition time. A similar approach was published by Mulder and coworkers [53]. The obtained results in this work are consistent with previously findings that show uptake of immunomicelles in cultured macrophages and in ex vivo atherosclerotic aorta [52]. Subsequently, Briley-Saebo et al. [54] conducted a study using micelles containing Gd and antibody (murine or human) that bind oxidation-specific epitopes (OSE). The aim of this work was to obtain a non-invasive in vivo imaging of atherosclerotic plaques rich of OSE by the use of MRI. Also in this case, the results show that the active targeting allows to obtain a significant signal enhancement using

micelles containing a specific antibody and a good identification of atherosclerotic lesions. In another work [55], the same authors changed the model previously adopted in order to evaluate the in vivo MRI efficacy of manganese (Mn(II)) as molecular imaging probe for OSE. Mn is a paramagnetic metal ion, endogenous, and bio-compatible and DTPA is used as the chelating agent. The intracellular accumulation in intraplaque macrophages of targeted bio-compatible Mn-micelles and de-metallation resulting in free Mn resulted in significant efficacy of contrast-enhanced MR imaging, allowing the visualization of atherosclerotic lesion through a non-invasive method.

Liposomes

Liposomes represent a delivery vehicle for active molecules (ie. imaging agents) and phospholipids are the main their constituent [56,57]. These amphiphilic molecules, consisting of a hydrophobic tail and hydrophilic head groups, confer to liposomes the ability to organize into spherical bilayer orientations in aqueous media. Generally, they contain an aqueous core and are used to encapsulate hydrophilic molecules [58]. Liposomes can be classified according to their lamellarity (uni-, oligo-, and multi-lamellar conformation), size, surface charge and preparation method [58,59]. These vesicular structures have been established for their passively and actively targeted applications, but they tend to accumulate in the liver and spleen due to recognition by RES. To avoid this phenomenon, researchers on liposome technology have progressed from traditional vesicles ("first-generation") to "second-generation", in which long-circulating time and stealth property are obtained by addition of polyethylene glycol (PEG) to the liposomes surface (process called PEGylation) [60,61].Because of the versatile possibilities of surface-modifications and active molecules encapsulation, liposomes have been extensively studied for delivery of bioactive agents in atherosclerotic lesions. Two approaches have been used to prepare liposome-based CAs: (1) encapsulation of the contrast agent into the liposome and (2) chemical conjugation of the MRI probe to the liposome membrane. An example of liposomes used for delivery to atherosclerotic tissue has been reported by Maiseyeu et al. [62], where Gd-decorated liposomes enriched with phosphatidylserine (PS) were used for imaging of accumulated macrophages at atherosclerotic site in ApoE -/- knockout mouse models. This approach allowed a significant enhancement of atherosclerotic plaque in vivo for molecular characterization of high-risk plaques. Based on similar rationale of macrophage activity in atherosclerotic lesions, Resen et al. [63] and Mulder and coworkers [64] have reported the development and contrast-enhanced targeted MR imaging of vascular disease associated inflammation using Gd-liposomes.

Dendrimers

Dendrimers are a highly significant class of nanosystems that exhibits many attractive characteristics and plays an important roles not only as drug delivery carriers, but also as imaging agents [65]. In more detail, they are nano-sized structures characterized by a controllable multibranched three-dimensional arrangement, globular shape, high functionality, small size and low polydispersity [66]. These structures offer three points for modification with diagnostic agents: the core, the branching zone and the branch extremities [67]. Therefore, active molecules may be encapsulated into the interior area or chemically/physically linked onto the nanovector surface. [68]. Their pharmacokinetics and pharmacodynamics features are not very clear and thus remain to be explored for their bioapplication [66]. In addition, the composition and size of dendrimer-based MR imaging agents influences their behavior. The pioneers in this field are Kobayashi et al. [69] that conducted a study about optimization of the performance of dendrimer-based MRI agents in vivo in comparison to Gd-[DTPA] using the poly (amido amine) (PAMAM) and diaminobutane core polyaminoamine (DAB) for the preparation of MRI contrast agents. They observed that dendrimer-based MRI contrast agents are quickly excreted by the kidneys and also able to visualize vascular structures better than Gd-DTPA due to less extravasation. Therefore, these structures are retained in the body for a prolonged time. Recently, Nguyen and colleagues [70] have synthesized, characterized, and evaluated the MR efficacy of manganese (Mn) dendrimers targeted to OSE in murine models. Considering that dendrimers can be easily modified to allow for the addition of contrast agents and antibodies for targeted delivery, PAMAM-based dendrimers were chosen for their ability to load large amounts of Mn and DTPA is chosen as chelating agent. The results demonstrated that the administration of the targeted dendrimers allow to obtain a significant enhancement of vascular lesions in comparison to untargeted dendrimers. The analysis was only qualitative because the observed MR imaging signal did not correlate with the histological presence of OSE.

CONCLUSIONS

Despite the progress in primary and secondary prevention and the growth of the knowledge base of atherosclerosis pathology, the incidence of myocardial infarction and stroke continues to remain high. Nowadays, the nanotechnology and the design of nanoscale devices seem to be a promising avenue for improving cardiovascular outcomes. The examples reported in this work include the use of NPs for MRI as tool for non-invasively evaluating atherosclerotic plaques, but their application in atherosclerotic field is very limited so far. A future goal in this field is represented by the combination of disease-specific biomarkers linked to the suitable carriers with MRI imaging modality in order to improve diagnosis and therapy of the atherosclerotic lesion. Therefore, it is essential to broaden our current understanding of distinct stages of pathological process for the development of novel diagnostic approaches based on these concepts. In the end, potentially harmful effects of these new methodologies must be borne in mind.

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